Memorial Sloan Kettering Cancer Center

Post-sort Purity Analysis SOP

Pictures from manufacturers' websites.

Flow Cytometry Core Facility

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We recommend that whenever possible, analyze the sorted cells to determine the effectiveness of the sort. The post-sort analysis data may be required if you want to publish the results from your sorting experiment.

Workflow Overview

Clean sample line ▶ Aliquot from collection tube ▶ Re-Add viability dye ▶ Acquire & record data ▶ Analyze the post-sort purity data

1. Clean sample line

1. Once the sorting is completed, unload the sample tube.
2. Load a tube containing RINSE (detergent) and run at flow rate 11 for at least 2 min, repeat the same procedure with DI water.

A very good cleaning is important to ensure reliable post-sort purity data.

2. Aliquot from collection tube

1. Move and cap the collection tubes from the sorting chamber.
2. Power on the tissue culture hood and clean the working platform with 70% ethanol.
3. Inside the tissue culture hood, mix the tubes well and aliquot 50µl from each tube.

3. Re-add viability dye

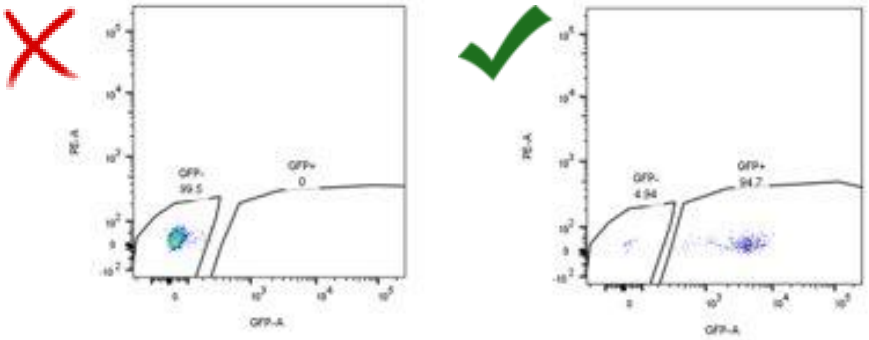
1. Re-add viability dye to each aliquot and mix well by gentle vortexing. Excluding the cells that died during the sorting from your analysis ensures a more accurate purity check.

4. Acquire and record data

1. Create a new tube under the same experiment and rename it with "sample name+population+ post-sort purity.
2. Load the aliquot tube.
3. Start acquiring and recording data at low flow rate 1-2.
4. Clean the sample line between samples by running RINSE (detergent) at flow rate 11 for 1 min, repeat with DI water.
5. Repeat 1 2 3 4 for all aliquots.

5. Analyze the post-sort purity data

1. Go through all recorded aliquots data in the global worksheet and note the percentage of those cells in the specific sorting gate accordingly.



Examples of a bad (✗) and a good (✓) sort purity check, after sorting GFP+ cells.

Some tips for improving post-sort purity

1. Successful instrument set up and QC is the good starting point for high purity sorting outcome.
2. Optimize the nozzle size. Large size nozzle such as 100 µm or 130 µm should be used for fragile cells.
3. *Doublets* discrimination gates strategy are crucial.
4. Pre-coat the collection tubes and filled them (1/4 full) with the appropriated medium.
5. Filter samples carefully with 40 µm strainer before loading.
6. Be careful during sample preparation to reduce clumping and fanning.